15:53:34 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 09/02/93

Active

Project #: G-32-640 Cost share #: Rev #: 1
Center # : 10/24-6-R7459-2A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R29 AI32880-02 Mod #: CARRY FORWARD Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA: 93.856
Main project #: PE #:

Project unit: BIOLOGY Unit code: 02.010.134
Project director(s):
BOHAN C A BIOLOGY (404)894-3700

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 930701 to 940630 (performance) 940930 (reports)

Sponsor amount	New this change	Total to date
Contract value	26,779.40	131,069.40
Funded	26,779.40	131,069.40
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: ROLE OF TAT IN HIV TRANSCIPTION INITIATION

PROJECT ADMINISTRATION DATA

OCA contact: E. Faith Gleason 894-4820

Sponsor technical contact

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MARY LEDFORD (OR TIM-MALA)
(301)496-7075

SAME

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
CARRY FORWARD OF $26,779.40 UNOBLIGATED FUNDS FROM 1ST YR OF GRANT (G-32-613)

SR100

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 09/27/93

Project No. G-32-640_____ Center No. 10/24-6-R7459-2A0_

Project Director BOHAN C A_____ School/Lab BIOLOGY_____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____

Contract/Grant No. 5 R29 AI32880-02_____ Contract Entity GTRC

Prime Contract No. _____

Title ROLE OF TAT IN HIV TRANSCIPTION INITIATION_____

Effective Completion Date 940630 (Performance) 940930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	_____
Final Report of Inventions and/or Subcontracts	Y	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

CommentsEFFECTIVE DATE 7-1-93. CONTRACT VALUE $131,069.40_____

Subproject Under Main Project No. _____

Continues Project No. G-32-613_____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other CARL BAXTER-FMD_____	Y
FRED CAIN-OOD_____	Y

NOTE: Final Patent Questionnaire sent to PDPI.

FINAL REPORT

Grant number: AI32880-02
Project number: G-32-640
Principal investigator: Cindy A. Bohan
Organization: Georgia Institute of Technology
Project Title: The Role of Tat in HIV Transcription Initiation

1. Specific Aims

HIV-1 Tat is unique among eukaryotic transcription transactivators by virtue of its ability to bind specifically to TAR RNA sequences and to activate both initiation and elongation events during HIV transcription. The general objective of this proposal is to elucidate the molecular mechanisms of Tat transactivation during transcription initiation. Localization of discrete Tat functional domains active in transcription initiation, dissection of the mechanism(s) by which Tat enhances preinitiation complex formation during HIV transcription, and determination of Tat-mediated effects on the synthetic step of transcription initiation are the specific aims of this proposal.

Aim 1: To localize discrete Tat functional domains active in transcription initiation.

(a) by site-directed and deletion mutagenesis of Tat and by evaluation of Tat mutants on HIV transcription at different stages of preinitiation and initiation

(b) by construction and evaluation of Gal4/Tat fusion proteins for their effect on HIV transcription in vivo and in vitro

Aim 2: To dissect the mechanism(s) by which Tat stimulates the assembly of preinitiation complexes during HIV transcription.

(a) by determination of association and disassociation rates of preinitiation complex assembly using gel shift and footprinting assays

(b) by determination of Tat-protein interactions using coimmunoprecipitation analysis, cosedimentation analysis, protein filter binding and Tat-affinity chromatography

(c) by determination of Tat effects on functional activities of preinitiation complex components

Aim 3: To determine whether Tat affects the kinetics of productive initiation complex formation.

2. Studies and Results

Aim 1: To localize discrete Tat functional domains active in transcription initiation

During the last and current budget periods, we have obtained from collaborators and/or generated 25 site-directed and deletion mutant Tat cDNAs. The mutations span regions within Tat that have previously been implicated in Tat-specific transactivation of HIV LTR-directed gene expression in vivo. The wt and mutant Tat cDNAs

have been subcloned into eukaryotic (pCMV) and prokaryotic (pet 25b) expression vectors. These recombinant clones have been confirmed by dideoxynucleotide sequencing. To characterize the specific Tat mutants for their ability to transactivate HIV-LTR-directed gene expression in vivo, wt and mutant Tat expression vectors have been cotransfected with an HIV LTR-CAT reporter plasmid into HeLa and Jurkat cells by calcium-phosphate precipitation and electroporation techniques, respectively. As predicted, the Tat domains encompassing the acidic, cysteine-rich and basic regions are required for transactivation of HIV LTR-directed expression of the CAT gene. Although these initial studies offer little novel revelations on the mechanism of Tat transactivation at the level of transcription initiation, they are **essential** for establishing a comparative means of characterizing wt and mutant Tat recombinant proteins that we are in the process of purifying from E. coli. These recombinant proteins used in in vitro transcription assays outlined within the grant should be helpful in identifying putative initiation- and elongation-specific functional domains in Tat.

Aim 2: To dissect the mechanism(s) by which Tat stimulates the assembly of preinitiation complexes during HIV transcription

Recently, we published data (presented as preliminary studies for the original grant proposal) revealing Tat's role in facilitating the initial stages of HIV transcription preinitiation complex formation. Subsequent and confirmatory to our initial observations, Tat has been shown to interact directly with TFIID and to stabilize HIV LTR-specific TFIID-TATA binding in vitro (J. Brady, personal communications). During the last and current budget periods, we have focused our efforts to establish mechanisms to determine whether or not the interaction of Tat with core transcription initiation complex components, such as TFIID, is a **functional** association. Anticipating their efficacy in retrieving active transcription preinitiation complexes in vitro that may be qualitatively and quantitatively characterized, we standardized the use of biotinylated HIV LTR templates in in vitro transcription assays and found that the 5'-biotinylated HIV LTR templates efficiently supported fully active transcription in nuclear run-off analyses.

Considering the possible differential involvement of the TATA, initiator (Inr) and TAR elements within the HIV LTR in the formation of Tat-responsive and Tat-unresponsive transcription preinitiation complexes, we have begun extentive mutagenesis of the LTR within these regions. These mutant LTR constructs, tested both in vivo and in vitro for their responsiveness to Tat-specific transactivation, should prove to be invaluable for defining differences in basal versus activated preinitiation complexes and their respective efficiencies to convert to processive elongation-competent transcription complexes.

Aim 3: To determine whether Tat affects the kinetics of productive initiation complex formation.

Conversion of transcription preinitiation complexes to actively initiated complexes is a multi-step process. One such step, which has been described for some promoters, is the phosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II (RNAPII). During the last and current budget periods, we set forth to determine ultimately whether or not Tat affects, indirectly or directly, the phosphorylation of the CTD of RNAPII and thus potentiates enhanced conversion of HIV-specific transcription preinitiation to actively initiated, elongation-competent complexes. In our initial attempts to standardize the methodology, we immunoprecipitated RNAPII (using CTD-specific antibodies) from HIV-specific basal transcription complexes that were synthesized in the presence of Hela cell-free extracts and P-ATP and that were stalled selectively at preinitiation and after active initiation to assess qualitative alterations in the phosphorylation patterns of the CTD during transition from preinitiation to active initiation. These experiments proved to be unsuccessful as abundant non-specifically phosphorylated proteins present in the immunoprecipitate caused extremely high background interference. To overcome this technical problem, we have been standardizing the use of biotinylated promoter templates in in vitro transcription assays to isolate initially only those proteins associated stably with the transcription complexes prior to phosphorylation and immunoprecipitation. Although no conclusive results may be reported to date, these templates have proved to be useful in reducing background interference.

3. Significance

The genome of human immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS), encodes, in addition to the common retroviral structural gene products, two essential regulatory proteins Tat and Rev. Tat, a 16-kd RNA-binding protein is localized primarily to the nucleolus and acts as a potent activator of HIV long terminal repeat (LTR)-directed gene expression to increase the steady-state levels of all HIV mRNAs. Since Tat activates HIV transcription at the levels of initiation and elongation, clearly understanding how Tat accomplishes either or both activities in the context of the integrated proviral genomes in latent and productive infections would be significant to the molecular biology of both eukaryotic transcription and retroviruses. Characterization of the role of Tat in the initial steps of transcription initiation and the interactions of Tat with basal transcription factors and/or cellular transactivator proteins as presented in this proposal should foster refinement of the complex biochemical nature of gene transcription. Further, these studies should illuminate the role of Tat in HIV gene regulation and provide more insight into the dynamics of Tat and cellular processes in the establishment and maintenance of HIV chronic latent and acute productive infections.

4. Plans

Aim 1: To localize discrete Tat functional domains active in transcription initiation

During the next budget period, we will continue characterizing the HPLC-purified recombinant Tat mutant proteins for their biological activities, including stimulation of HIV LTR-directed gene expression in vivo and of AIDS-associated Kaposi's Sarcoma (KS) cell growth, as well as for their abilities to facilitate HIV-specific preinitiation complex assembly in vitro. Additionally, we plan to construct and evaluate GAL4/Tat fusion proteins with LTR and derivative templates for their effect on transcription in vivo and in vitro. The LTR mutants that we are preparing currently will be useful in these studies.

Aim 2: Dissection of the mechanism by which Tat stimulates the assembly of preinitiation complexes during HIV transcription

Mutant HIV LTR templates, currently being generated, will be added to in vitro transcription reactions containing 0.015% sarkosyl in the presence or absence of Tat to identify promoter sequences that are required for both basal and Tat-responsive preinitiation complex formation. To determine whether Tat enhances the stability of the formed preinitiation complexes and, therefore, increases the effective concentration of complexes available for productive initiation, pulse-chase experiments using preinitiation complexes preformed on biotinylated and radiolabeled LTR templates and excess amounts of cold HIV LTR DNA will be performed in the absence or presence of Tat. Aliquots of preformed preinitiation complexes will be removed and purified at various times following addition of excess wt and mutant LTR DNAs and separated on native polyacrylamide gels. Dissociation rates for these gel-shifted complexes will be determined to assess the qualitative and quantitative effects of Tat on complex assembly and complex stability.

Following in vivo and in vitro characterization of wt and mutant recombinant Tat proteins, we will begin investigating whether a direct interaction between Tat and components of preinitiation complexes exists by Tat-affinity chromatography. Should such an interaction be demonstrated, we will pursue testing the functionality of the interaction as outlined within the original proposal, utilizing the biotinylated HIV LTR templates standardized during this current budget period.

Aim 3: Determination of whether Tat affects the kinetics of productive initiation complex formation

We plan to continue our efforts towards determining whether phosphorylation of RNA polymerase CTD is an essential event in HIV transcription. If CTD phosphorylation proves to be required for efficient conversion of HIV preinitiation complexes to active initiation complexes that are fully processive, we will perform

experiments, standardized during this current budget year and presented above, in the presence or absence of Tat to examine whether Tat mediates an effect on CTD phosphorylation.

5. Human Subjects

None

6. Vertebrate Animals

We obtained an IACAUC control number (03-93) from the Institute Animal Care and Use Committee at Georgia Tech (Chair: Jim Toler) July 14, 1993 for the purpose of generating epitope-specific anti-Tat monoclonal antibodies in mice. All animal work was performed by Dr. John Morrow at Texas Tech University Health Sciences Center in Lubbock, TX under a consortium/contractual agreement. The work for this contract was completed September 1, 1993.

7. Publications

Bohan CA, Kashanchi F, Ensoli B, Buonaguro L, Boris-Lawrie KA and Brady JN. 1992. Analysis of Tat transactivation of human immunodeficiency virus transcription in vitro. Gene Expression 2: 391-407.

8. Inventions and Patents

None

FINAL BUDGET REPORT

Grant number: AI32880-02
Project number: G-32-640
Principal investigator: Cindy A. Bohan
Organization: Georgia Institute of Technology
Project title: The Role of Tat in HIV Transcription Initiation

DIRECT COSTS:	Beginning funds	Expenditures	Remaining funds
Personnel Services	$66,727	$26,718	$40,009
Fringe Benefits	$10,197	$4,611	$5,586
Travel	$2,000	0	$2,000
M&S	$13,979	$4,071	$9,908
Equipment	0	0	0
Subcontract	$10,000	$10,000	0

The remaining funds will be transferred to Tulane University Medical Center.